Reevaluation of Microbial Water Quality:

Powerful New Tools for Detection and Risk Assessment

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14. ABSTRACT

This report outlines the background and need for new tools to deal with issues of water quality. The recommendations, provided by a colloquium held by the American Academy of Microbiology, include information about training, new tools and needed research.

15. SUBJECT TERMS
Water quality, microbiology, water safety, molecular tools

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In the dead of night, a storm rumbles across farmlands and cow pastures; torrents of rain drench the earth. Of the hundreds of cows that graze in the saturated fields, several harbor E. coli O157:H7, a bacterium harmless to livestock but potentially deadly to people. The bacterium, shed in the cows' manure, is washed into a stream that feeds a public water district 20 miles away.

Hours later, a red light flashes on an electronic watershed map mounted on the control panel of the water district's monitoring station. Microbial contaminants—detected by gene chips affixed to stationary stream posts (or implanted in the gills of fish sentinels) and inserted into wells—have entered the system. Other lights flash, indicating the identity of the microbe. The lights alert the district water manager. She tracks the contaminants. Noting that virulent E. coli bacteria are moving into the town's shallow well system located at the river banks, she ratchets up the chlorination, and then contacts the regional health manager who issues a boil water order. The next morning, local TV and radio stations issue the warning, and local citizens, especially those with young children, hospitals, and the elderly are all alerted. A possible E. coli outbreak is averted. The new water quality monitoring system has passed a rigorous test. Nature designed the test. The water district and the residents of the town knew it would come, they just didn't know when or where.

In this scene from the future, gene chip technology identifies a target microorganism and registers its presence in real time through telemetry. The miniature chip contains "capture genes," genetic sequences specific to dozens of different pathogenic microorganisms. When the target genetic material contacts the chip, it produces an electronic signal alerting the water management facility. The chip is just one of a number of technologies being developed in universities and laboratories around the country to assure water quality. When these technologies are available, they will save lives and prevent illness caused by waterborne disease. For now, however, water quality monitoring is mired in the past. This past has served the public well, but it is time to move ahead to better protect public health.

The Need for New Tools

For more than 100 years, the water industry has depended on methods that detect and count "indicator" bacteria by exposing water samples to nutrients, incubating the samples, and encouraging growth of bacteria that usually thrive in the human colon. If these "coliform" bacteria grow, they must have come from feces, and, therefore, the water must be contaminated. The rules that a good "indicator" organism should follow, while sound in principle, cannot be met by the current coliform bacterial indicator system. The many flaws in the current techniques make this indicator system unreliable. The test misses or fails to "indicate" disease-causing viruses—such as Hepatitis A or E, Coxsackie viruses, Adenoviruses and Norwalk viruses and indigenous pathogenic bacteria, such as *Heliocobacter* or *Legionella*, as well as parasites *Cryptosporidium* and *Giardia*. Many of these organisms are currently on the U.S. Environmental Protection Agency (EPA) "Contaminant Candidate List" and cannot afford to be missed. In addition, it has long been known that when coliform testing is used to evaluate water purity after disinfection, contamination can be and has been missed.

Outdated detection methods do nothing to identify and prevent serious global enteric waterborne disease from which over 2 million children die annually (WHO, 2000). Newly identified microbes, such as the nanobacteria, unknown to many scientists, are ubiquitous in drinking water, yet the diseases they cause are not well established. There are as-yet-unidentified microbes that have been suspected to cause human disease, but for which culturing methods have not yet been developed. In the last decade, problematic changes have occurred, such as emerging antibiotic resistant bacteria, often found in water downstream from animal farms. Yet no good detection methods have been developed for addressing this trend. Current methods for testing water clearly do not adequately assess

the risk of waterborne disease, water quality, or even treatment needs.

Advances in microbiology have impacted every field of science, medicine, agriculture, bioremediation, space science, and defense technology (e.g., biological weapons). Specific, rapid, sophisticated methodologies are available, yet in water science and technology, industry and government have failed to support or take advantage of these new and powerful techniques.

Nature and Scope of the Problem

Microbial water quality detection methods have become outdated, at a time when the risks to water supplies appear to be increasing. Because the hydrologic system is interconnected, water managers must be concerned with the quality of water used for all purposes—sustaining life, communities, and economies. Rainwater, surface water, ground water, and coastal and beach waters are all interconnected, and the water supply itself is connected to the food supply. National headlines report every year more swimming and fishing areas closed and more people getting sick from the water they drink.

CONTAMINATED WELL WATER. On May 23, 2000, what many people in Walkerton, Ontario, suspected was confirmed. The town's well water was contaminated with *E. coli* O157:H7. As of July 10, the outbreak had killed seven people and was being investigated in nine more deaths. Scores more had fallen ill—90 people had been admitted to the hospital, almost 1,000 were treated and released, and another 1,000 sought medical phone advice for treating diarrhea or cramps. Some children suffered permanent organ damage. Health officials believe that heavy rain had washed manure contaminated with the *E. coli* into wells at a time when the chlorination system was broken. Monitoring results were not available for at least 24 hours, and officials were slow to respond since the nature of the threat was not identified (Canada Communicable Disease Report, Oct. 2000).

BEACH CLOSURES. For two months during the summer of 1999, the beaches were closed in Huntington Beach, California, due to high levels of enterococci and coliform bacteria, indicating high levels of contamination and potential risk to anyone using the beach. Local businesses suffered millions of dollars in lost revenue, and each day 30,000-40,000 beach goers had to forego their summer recreation. Officials pursued a number of suspects—a construction site where dredging material was illegally discharged into a storm drain, leaks in the sewage system, as well as storm waters discharged through wetlands. The source was never identified (Lemus and Weisberg, 2000).

Nationwide, 729 beaches were closed for at least one day in the summer of 1998. In total, more than 7,000 beach days were lost, mostly in New Jersey, California, and Florida. Almost every coastal and Great Lakes state reported having at least one beach where storm water was a known source of pollution at or near bathing beaches (National Resource Defense Council, 1999).

DEATHS FROM A CONTAMINATED WATER PARK. On June 11, 1998, hundreds of children playing in a water theme park in suburban Atlanta were exposed to a deadly strain of *E. coli*. Two children died of kidney failure and other complications; another 24 became ill. The Georgia Division of Public Health identified the source as fecal contamination and insufficient chlorine levels. Health investigators later genetically matched an unusual strain of *E. coli* O157:H7 found in the water to that in contaminated beef distributed by Bauer Meat Co. in Ocala, Florida. Before being recalled, a third of the beef had gone to Georgia (Gilbert and Blake, 1998).

CONTAMINATED SHELLFISH. In June 1998, 367 people who had eaten raw oysters in restaurants from Florida to California became ill with diarrhea, nausea, vomiting, and stomach pain. Americans had spent more than $11 million a year on Texas oysters, and that supply was suddenly suspect. To prevent further illness, oystering in Galveston Bay, Texas, was shut down for four months.

In July of 1998, the Centers for Disease Control and Prevention ran a test on a stool sample and found a virulent serotype of a common bacterium, *Vibrio parahaemolyticus*. The strain, O3:K6, common in Southeast Asian waters, had not been reported in the United States since 1972. Although scientists widely tested Galveston Bay oysters for the strain, they never found it. In the meantime, oystermen mortgaged their homes and risked bankruptcy (Barwick, et. al., 2000).

Flooding, pollution, or sewage runoff can elevate bacteria or virus levels in oyster habitats, and, since oysters are filter feeders, these bivalves can become pathogen reservoirs. Enteric pathogenic viruses were found in coastal waters and shellfish harvesting waters in the absence of indicator bacteria and were highly associated with rainfall and non-point sources of pollution, such as septic tanks (Lipp, et. al., 2001). This type of pollution also changes the ecological nature of the microorganisms that reside in these waters, affecting fish and the fishing industry. Bacteria, such as *Aeromonas* and *Vibrio*, cyanobacteria, and *Phiesteria*, may bloom and, as a result, affect fresh waters, estuarine, and coastal waters, thus affecting the health of the fish and even humans (Grimes, 1991).

In the future, water quantity will be an issue, as well as water quality. The challenge will be to have enough water where and when it is needed. As the human population increases, so too do coastal populations. Nearly two-thirds of humanity already resides within a 50-

mile radius of a coast, often in close proximity to poorly treated sewage discharges. Coastal waters increasingly are relied upon for harvesting shellfish and finfish raised in aquaculture to supplement dwindling fish catches from the oceans.

Moreover, the movement in human populations from one region to another has increased over the past 20 years, and new transportation routes easily reach what were once remote destinations. The speed of human movement has increased as well. With many more people relying on air travel that takes them thousands of miles in a matter of hours, pathogens can reach new hosts with jet-set speed. Increasing globalization of trade also increases risks. Scallops caught off Scotland are sold in Australia; oysters raised off the coast of South America are sold in North America. In some cases, such aquaculture products have been documented to disperse harmful microbes to distant regions of the world. World trade organizations and agreements, such as the North American Free Trade Agreement (NAFTA), have suggested that food, as it enters the global food market, should have an equivalent level of microbial safety. This implies that the presence of pathogenic microbes in fish or shellfish in unacceptable, yet no approach for monitoring has been implemented.

Governments, utilities, and communities will have to find the right balance among treating water, protecting watersheds, and enabling the full spectrum of human water uses. New monitoring tools will help us reach that balance in a cost-effective way while protecting human health. New techniques will aid development of strong early warning systems, reliable field diagnostics, symptom treatments, and more effective remediation of impacts from harmful microorganisms. New technologies have an exciting future—but only if we invest in them.

Microbial Risk Assessment

Investment should begin with improving microbial risk assessment techniques. Risk assessment is the process of integrating scientific data regarding an environmental hazard into a framework to address the risk of exposure and the potential health impacts. This process has proven invaluable to the regulatory community, industry, and risk managers who must conduct comprehensive evaluations of everything from total maximum daily loads (TMDLs) to a watershed to viruses in groundwater to shellfish and fish safety associated with the global fish market.

Biologists have only recently begun to use this methodology to examine the risks microorganisms pose through contaminated drinking water, recreational water, or water in which shellfish is harvested. Through developing microbial risk assessments, scientists not only identify potential health hazards, but also can determine the adequacy of the underlying data. A risk assessment allows scientists to zero in on the data gaps most critical to improving the precision of their assessment. Unfortunately, those gaps are large and many. Risk assessments have generally been poor, due to a lack of information on everything from the prevalence of specific microorganisms, to their health effects, and their genetic code.

For most waterborne pathogenic microorganisms, historical databases on occurrence in water are sparse or lacking. The general public fully understands vacation diarrheal episodes, but there is little or no data available on the sources of these episodes, the type, or extent of waterborne diseases. Ocean-going tourists, for example, suffer episodes of diarrheal diseases. The all-too-frequent cases of such diseases are attributed to overindulgence in rich foods, when more probably contaminated coastal waters or seafood are the culprits. All of the microorganisms currently on the EPA's "Contaminant Candidate List" lack a database, thus no assessment on exposure can be made. The List has been prepared to address emerging risks to water, and a new list should be developed every five years. An adequate risk assessment needs to address both health risks and exposure. It is anticipated that only one of the microbes on the current list will be addressed by the time a new list is developed.

BACTERIA	PROTOZOA	VIRUSES
Aeromonas	*Ancanthamoeba*	Adenoviruses
Cyanobacteria	Microsporidia	Caliciviruses
Helicobacter	(*Enterocytozoon* and	Coxsackie Viruses
Mycobacterium Avium intracellulare	*Septata*)	Echovirus

EPA MICROBIOLOGICAL CONTAMINANT CANDIDATE LIST

The coupling of risk assessment with microbial monitoring information may require advances in the Microbial Risk Assessment framework. For instance, there is increasing evidence that diverse microbial species exchange genetic elements responsible for pathogenicity. If so, risk control would benefit from a better understanding of the occurrence of these genetic elements, the dynamics of the genetic exchange, and the gene sequences themselves.

The integration of the risk assessment framework and the application and development of new tools is shown in Figure 1. One may enter the risk framework through the disease pathway or the detection of the microbe in water or suspicion that the microbe is in water. While culture techniques have been the focus of environmental microbiology, it is clear that this approach does not allow for complete characterization of risks; it also delays characterization. Culture—in and of itself—is a minor and nonessential component of the approaches necessary for risk assessment.

The conventional risk framework includes:

- Hazard identification (types of pathogens and description of illnesses, hospitalization, and mortality).
- Dose-response (quantitative relationship between dose and outcome, e.g., ID50, the number of microbes required to initiate infection in 50% of the exposed population).
- Exposure assessment (prevalence, concentrations, distribution in time and space in water or food consumed).
- Risk characterization (the quantitative likelihood of potential adverse health outcome based on the above).

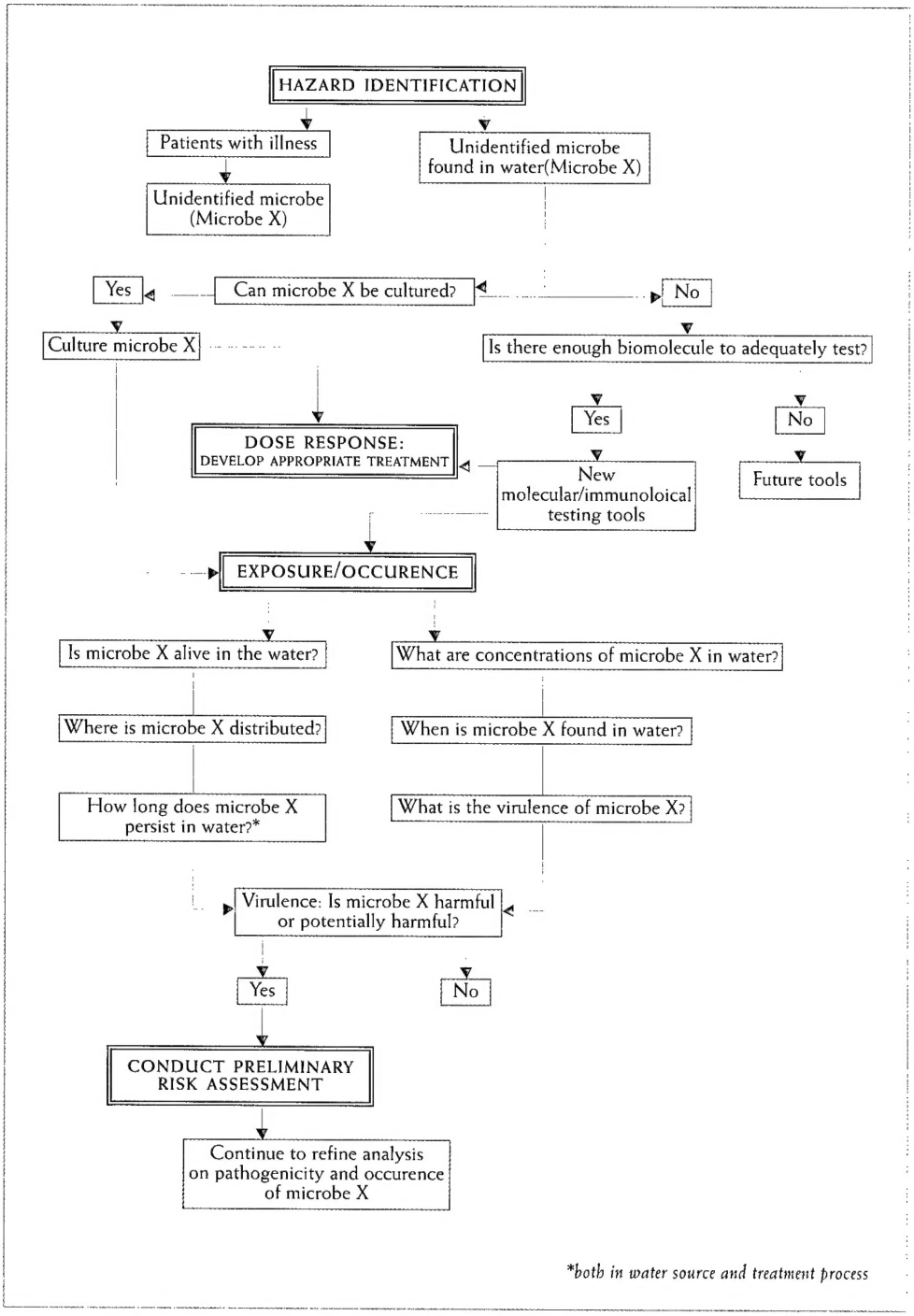
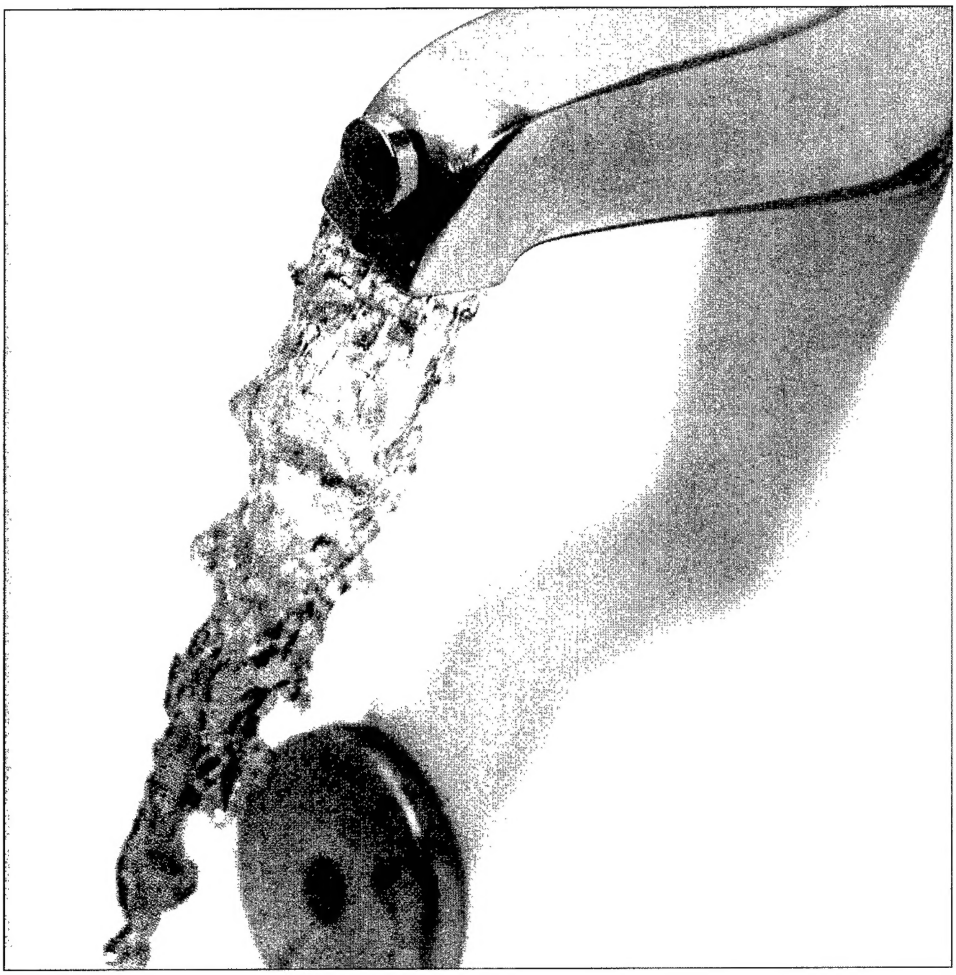


FIGURE 1. INTEGRATED TOOLS AND RISK ASSESSMENT FRAMEWORK



Many waterborne microorganisms are not culturable or are inefficiently cultured. This includes many viruses and bacteria, such as *Legionella* and *Helicobacter*. In addition, waterborne bacteria may transition from an easily culturable state to a nonculturable state of existence, thereby falsely suggesting that they have died (Colwell and Grimes, 2000). Thus, the immunological and, in particular, the genetic/molecular methods provide an immediate approach that can be used to gather necessary data on occurrence, prevalence, virulence, and even viability and quantification in some cases. These methods and data will add not only to the exposure assessment, but to the hazard identification and dose-response assessment.

Emerging risks could be evaluated, and risk management implemented. Risk, such as BSE and the possibility of waterborne transmission of prions, needs to be assessed. Because BSE can be transmitted through ingestion and is resistant to disinfection, it is likely through the disposal of animal wastes and byproducts that these prions are found in water and pose a public health risk. Antibiotic resistance and the risks of gene exchange accelerated through the water transmission route are of growing concern. There is increasing evidence of diverse microbial species exchanging genetic elements responsible for pathogenicity. If so, risk management would benefit from better understanding of the occurrence of these genetic elements, the dynamics of the genetic exchange, and the gene sequences themselves.

Risk assessment is an acceptable, scientifically based, and credible method for addressing environmental hazards and developing risk management methods that will protect public health. How-ever, the assessment of microbial waterborne disease risks cannot be approached without the addition of new tools.

RECOMMENDATIONS:

- Incorporate new tools into the risk assessment framework.
- Begin addressing risks, such as BSE and genetic resistance, pathogenicity, and gene exchange.
- Address the microorganisms on the "EPA Microbiological Contaminant Candidate List" using the new integrated risk assessment framework and tools.
- Formulate an international commission to address implementation of this approach worldwide.

New Molecular Tools

In the era of the Human Genome Project and the announcement of the complete sequencing of the genetic information that makes up a human being, it is clear that the tools for characterizing microorganisms exist. This genetic assessment also leads to knowledge of proteins and functions—in this case, the potential for causing waterborne disease.

Both genetic and immunological (protein based) tools are available and have been used to address waterborne pathogenic microorganisms.

GENE PROBES ARE PIECES OF DNA THAT ARE COMPLIMENTARY AND BIND TO KEY SEQUENCES. THESE CAN BE LABELED AND DETECT MICROORGANISMS OF SPECIFIC INTEREST. PCR (POLYMERASE CHAIN REACTION) IS A BIOLOGICAL COPYING SYSTEM THAT COPIES LOW NUMBERS OF SPECIFIC GENES SO THEY CAN BE DETECTED. ANTIBODIES ARE BIOLOGICAL MOLECULES THAT CAN BE PRODUCED AND LABELED FOR DETECTION. THESE BIND TO SPECIFIC PROTEINS ON A BACTERIAL CELL WALL, OR VIRUS CAPSID OR OOCYST/CYST WALL. THESE TECHNIQUES CAN BE ADAPTED TO INSTRUMENTATION, QUANTIFICATION, AND AREAS LIKE GENE CHIP TECHNOLOGY THAT ALLOWS FOR DETECTION OF MULTIPLE TARGETS AUTOMATICALLY.

Several university laboratories are using innovative new detection methods for indicator systems and pathogens. Many involve detection of telltale bacterial genes with "gene probes." A scientific group in North Carolina has a genotyping method for coliphages (viruses that infect *E. coli*), which is useful to distinguish animal from human. A group in Montana is using microscopic magnetic beads coated with an antibody against *E. coli* O157:H7 and a magnetic field to pull out the specific microorganism. Genes of viruses are first multiplied to detectable levels with the polymerase chain reaction (PCR), a biological copying process at the University of South Florida. Multiplex PCR systems for detecting *E. coli*, which could distinguish the pathogens from the normal *E. coli*, have been proposed by a group at the University of Alabama, Birmingham.

Molecular techniques are already used in clinical medicine and can be adapted for environmental testing. Of greatest interest are rapid, direct molecular tests that clinical laboratories routinely use to detect pathogens, such as the agents of tuberculosis and whooping cough in blood, feces, urine, and sputum. These methodologies are being adapted for environmental and food samples, and they show great promise for direct testing of water. Gene probes are being used that are highly specific and capable of detecting genetic sequences of DNA and RNA common to or conserved in pathogens such as *Salmonella* and *Legionella*, two disease-causing microbes. Researchers have now developed PCR, gene probe, and DNA "fingerprinting" techniques to detect intestinal bacteria and viruses in seawater and seafood.

Some molecular techniques are particularly promising as means to identify the source of a contaminant. Without source identification, contamination may not be contained. Such was the case in Huntington Beach, where, despite the expenditure of millions of dollars, the contaminant was never uncovered, and the beach remained closed for the remainder of the summer, until the situation ultimately resolved itself (Lemus and Weisberg, 2000). Molecular fingerprints, host-specific genes, and chemical constituents provide direct evidence of source origin, while general indicators provide virtually no information about source. On the most basic level of identifying the source of fecal contamination, scientists need to determine whether it is of human or other animal origin. To do so, they can use certain behavioral traits of the microorganisms. For example, antibiotic resistance profiles of bacteria can differentiate between human and non-human fecal sources, since the bacteria that infect people and livestock are often resistant to different antibiotics. However, there are numerous techniques being identified (Harwood, et. al., 2000).

Genotyping, or molecular characterization, is a powerful new tool for source identification of microbial contaminants. Molecular characterization of microbial contaminants in water typically consists first of concentrating and purifying the genetic material, followed by DNA/RNA extraction, and nucleic acid amplification. The amplified nucleic acid would then be analyzed by either restriction fragment length polymorphisms (RFLP) for patterns, restriction digest for presence/absence of specific sequences or sites, or DNA sequencing. Scientists regard DNA sequencing as the most discriminating method, able to distinguish between microbial strains because whole regions of the genome can be compared base-by-base.

New molecular methods for typing will assist in the identification process during outbreaks and contamination events. For example, scientists searched a national genetic database that catalogs all known genetic fingerprints of *E. coli* O157:H7. The search revealed that the *E. coli* found in a contaminated water park in Georgia was genetically identical to *E. coli* found in meat sold by a patty distributor in Florida (Gilbert and Blake, 1998). However, although the strain was highly unusual, scientists could not be certain that the tainted meat was the original source of the water park *E. coli*. The deadly strain could have entered the park through another means. Because *E. coli* infections are rarely genetically mapped—even when they are reported—some other unidentified contaminated food could have led to the fecal introduction of the water park *E. coli*. In contrast, during the Huntington Beach closure investigations that cost 2 million dollars, the source was never identified and no new techniques were utilized during this investigation

Many challenges exist. Specific gene target approaches rely upon often scanty knowledge of the frequency of the target gene within the host community. The trait's frequency will determine the sample volume needed to test for the microbe. Some tests use restriction fragment length polymorphisms to identify fecal organisms. The

THE FOOD AND DRUG ADMINISTRATION HAS BEEN EVALUATING SEVERAL PROTOCOLS THAT CAN BE ADAPTED FOR RAPID TESTING OF SHELLFISH FOR CALICIVIRUSES, NORWALK VIRUSES, AND SMALL ROUND STRUCTURED VIRUSES (SRSV). SINCE 1990, GREATER THAN 90% OF ILLNESSES ASSOCIATED WITH SHELLFISH CONSUMPTION IN THE U.S. HAS BEEN ATTRIBUTED TO SRSV (GLATZER, 1998). THE PROCEDURE UTILIZES AN APPROACH KNOWN AS RT-PCR (REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION) WHICH CAN DETECT RNA VIRUSES. VERY LOW LEVELS OF VIRUSES CAN BE DETECTED IN ABOUT EIGHT (8) HOURS, COMPARED TO SEVERAL WEEKS FOR TRADITIONAL TESTING METHODS. THE METHOD HAS BEEN USED TO DEMONSTRATE THE POTENTIAL HEALTH THREAT FROM CONTAMINATED SHELLFISH (ATMAR, ET. AL., 1995; ATMAR, ET. AL., 1996) AND HAS BEEN TESTED DURING EPIDEMIOLOGICAL INVESTIGATIONS AND OUTBREAKS (SHEIH, ET. AL., 2000; LEGUYADER, ET. AL., 1996). THE METHODS WILL BE JOINTLY TESTED AND THEN BE AVAILABLE IN SEVERAL KEY LABORATORIES AROUND THE COUNTRY.

accuracy of these tests requires databases that represent the genetic variation that naturally exists within the target population.

The vast majority of harmful aquatic microorganisms have not yet been sequenced. However, geneticists are sequencing new organisms at a rapid clip, and the sequences are commonly deposited in GenBank, the genetic sequence database of the National Institutes of Health. In June 2000, its annotated collection of all publicly available DNA sequences encompassed approximately 8,604,000,000 bases in 7,077,000 sequence records.

As useful as this resource is, problems remain. For instance, there is no peer review or validation of sequences that are incorporated into GenBank. Responsibility for corrections of sequence data falls to the submitting author, who may not even be aware that errors exist in the sequence. Errors may be introduced during genetic amplification, by cloning enzymes, base calling software, and chimeric PCR products. Criteria for generation, editing, and submission of new sequences need to be developed and disseminated to the scientific community. Insufficient oversight of deposited sequences compromises the quality of information available to other investigators. Guidance is needed because highly accurate genetic sequences are becoming ever more integral to the molecular techniques upon which future microbial risk assessment relies.

As these examples show, more resources are needed to increase the usefulness of many of the new molecular techniques. Genetic databases must be expanded. Scientists must establish test specificity, sensitivity, and stability, as well as potential temporal and spatial variations in the target. Yet, despite these challenges, new genetic techniques offer the best hope for comprehensive, accurate, and timely detection of microbial contaminants in water.

RECOMMENDATIONS:

- Enhance utilization and acceptance of new source-origin molecular fingerprinting techniques by establishing test specificity, sensitivity, and stability, as well as potential temporal and spatial variations in the target. Determine test performance with various types of samples.
- Build databases that represent heterogeneity of the characteristic among the target population. Address geographical limitations and consider whether new databases need to be developed for specific applications. Develop well-defined control strains to verify the accuracy of the test.
- Determine the frequency or prevalence of the target gene within the host community for specific gene target approaches to determine the sample volume needed to capture the required number of organisms containing the target trait. Incorporate interference controls.
- Apply highly discriminating techniques to identify etiological agents among heterogeneous microbial populations in water and seafood.

TOOL	CHARACTERISTICS AND ADVANTAGES	LIMITATIONS
Gene Probes	▪ Relatively rapid compared to conventional culture methods ▪ Can be used for quantitative assay, especially for microorganisms[1] ▪ Can differentiate agents carrying the known virulent genes and, thereby, differentiate potentially virulent strains from nonvirulent strains	▪ Only applicable to culturable microbes ▪ Cannot determine the infectivity of the microbe
PCR	▪ Applicable for detection of specific infective agents and their virulent genes; can target specific genetic elements; can be rapid and specific ▪ Can be used for quantitative assay for a limited number of pathogens[2] ▪ Infective agent does not have to be culturable for direct identification ▪ Can be used for identification of functionality of the virulent genetic element (RT-PCR) ▪ Can be applied to detect viruses that do not have a defined laboratory animal model ▪ Can easily be used with other viability methods (e.g., culture techniques)	▪ Only applicable if sufficient quantity of nucleic acids can be recovered from the targeted harmful microorganisms ▪ Inconsistencies in performance of this methodology can increase uncertainty of the technique or make it unreliable (in most applications) ▪ Must validate PCR methodologies (QA/QC) and "troubleshoot" to ensure reliability and optimal conditions prior to implementation ▪ Currently unable to discern viable from non-viable microorganisms
RAPD, AFLP, AP-PCR DNA Fingerprint Analyses	▪ Genetic fingerprints can be generated by PCR amplifications followed by, if necessary, restriction endonuclease treatment ▪ A disease-causing infectious agent can be traced for its source; this is helpful for discerning the occurrence, distribution, and prevalence of a specific disease-causing agent ▪ Pulsefield gel electrophoresis can also be useful[3]	▪ Currently unable to discern viable from non-viable microorganisms
BioSensors	▪ Immunoaffinity step to capture and concentrate bacteria on beads, membranes, or fiber optics probe tips, followed by detection of bound bacteria by laser excitation of bound fluorescent antibodies, acoustogravimetric wave transduction, or surface plasmon resonance ▪ Rapid, but must have culturable microorganisms	▪ Currently unable to discern viable from non-viable microbes
Immunomagnetic Capture Approach [4]	▪ Relatively specific for the targeted harmful microbe	▪ Sensitivity, consistence, and robustness for application across different environmental conditions ▪ Currently unable to discern viable from non-viable microbes
Gene Chip Technology	▪ Visionary approach currently being tested and modified by a group of biotechnology companies for use in microbial water quality ▪ 4-hour detection ▪ Sensitive to the desired level for certain harmful microorganisms ▪ Specific ▪ Being developed to be ten-fold less expensive for determining expressed genes in the environment	▪ Technique not yet available, so limitations cannot be determined
Solid-State Biochip	▪ Visionary approach currently being developed for the rapid detection (minutes) of a number of toxins and actual microbial cells ▪ Approach does not require isolation and characterization of the genetic elements ▪ No capturing of antibody ▪ No lengthy incubation times ▪ No labeling ▪ No washing	▪ Technique is not yet available, so limitations cannot be determined

[1] For example, *Vibrio vulnificus* or *V. parahaemolyticus* from oyster homogenates → enrich in suitable growth media → grow on agar plates → hybridize on filter, using specific gene probes for pathogenic and nonpathogenic strains → non-hazardous colorimetric signal identification → quantify the harmful microbes from the positive signals.

[2] For example, *E. coli* by TaqMan assay (PE).

[3] Note: These methods can produce inconsistent results unless they are first carefully optimized and validated.

[4] Harmful microorganisms can be captured from a complex environmental sample using magnetic beads coated with specific antibodies, followed by detection using gene probes and/or PCR methodologies.

The Utility of Indicators

Indicator microbes are used to predict and/or minimize the potential risk from pathogenic microbes. Indicator organisms are useful in that numerous pathogens may be transmitted via a water route. Indicators circumvent the need to assay for each and every pathogen. Ideally, indicators are rapidly detected, easily enumerated, and have survival characteristics that are similar to those of the pathogens of concern.

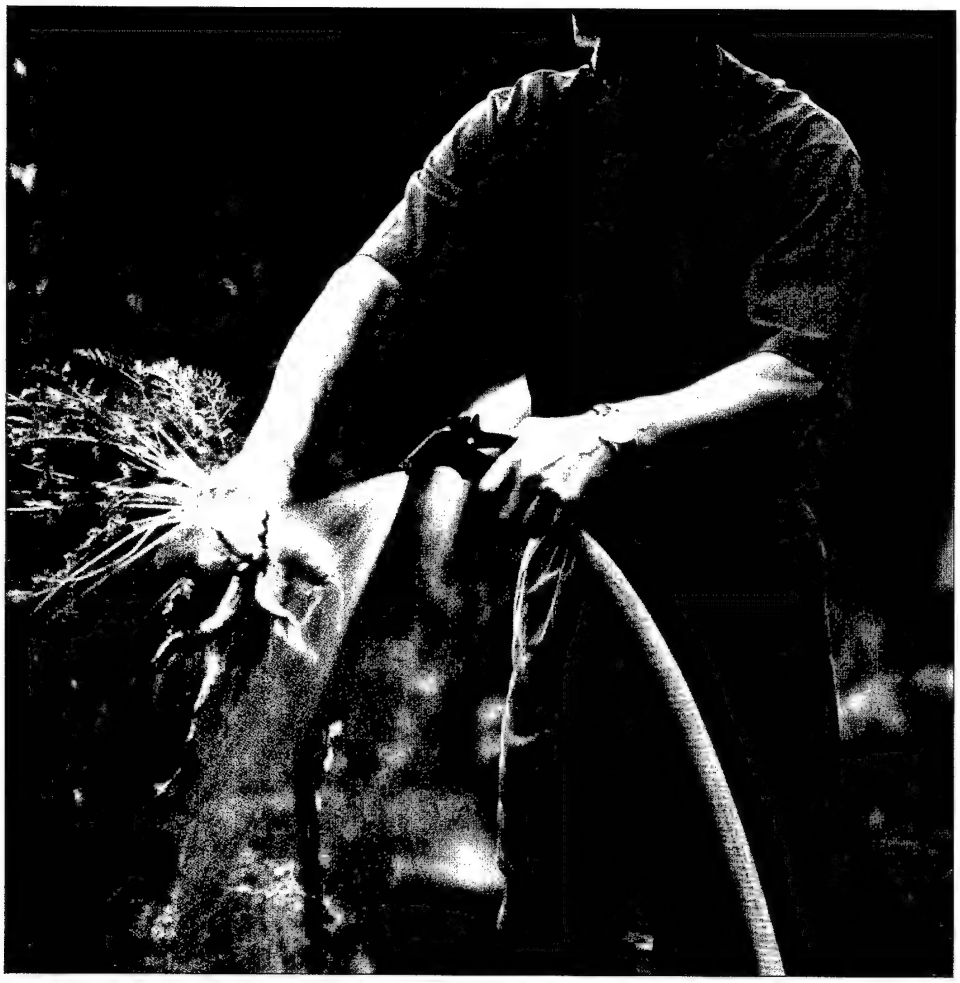
Fecal coliforms have been used extensively for many years as an indicator for determining the sanitary quality of surface, recreational, and shellfish growing waters. In recent years, scientists have learned more about the ways in which the coliforms' ecology, prevalence, and resistance to stress differs from many of the pathogenic microorganisms they are proxy for. These differences are so great that they limit the utility of fecal coliforms. Therefore, numerous alternative microbes have been suggested to play the role of indicator, including *E. coli*, enterococci, *Clostridium perfringens*, male-specific coliphages, and bifidobacteriaphages. Alternative chemical indices have also been suggested as complements to fecal coliforms. These include corprostinol or caffeine compounds. A drawback to these alternatives is that their ability to assess risk from water usage is unclear.

Before alternative indicators replace or augment fecal coliforms, their application must be specifically defined to ensure that they do a better job than the coliforms themselves in reflecting health risk.

INDICATOR	ADVANTAGES	CONCERNS
E. coli: more fecal-specific than fecal coliforms (*Klebsiella*) which have no true fecal link. Used by the European Union for assessing shellfish safety	Easily enumerated, rapid, low-tech	May not strengthen ability to identify risks of viruses and parasites relative to coliforms, rapid die-off rates in certain waters, and regrowth potential
Enterococci: have been used as an index of hazardous conditions from fecal contamination at bathing beaches	Ubiquitous in wastewater effluents	There are naturally occurring ("environmental") sources or reservoirs
Clostridium perfringens: has been used effectively as a tracer of sources of fecal pollution	extremely resistant to environmental stresses (e.g., temperature, salinity, uv/sunlight stress) and disinfection (e.g., ozone, chlorine)	Found in low numbers; dilution may be an issue. Anaerobes are difficult to cultivate
Male-specific bacteriophages: ubiquitous in wastewater effluents and resistant to disinfection	Associated with wastes from warm-blooded animals and share many characteristics in common with enteric viruses	Unstable in warm estuarine waters

EXAMPLES OF THE UTILITY OF ALTERNATE INDICATORS[5]

[5] Many of the alternate indicators that have been suggested to date require further development and evaluation before they can be reliably and widely implemented.



Crisis Management

By their nature, outbreaks of waterborne disease always come as a surprise. From 1997 to 1998, a surveillance program run by the Centers for Disease Control and Prevention (CDC) and the Environmental Protection Agency (EPA) reported 17 outbreaks associated with drinking water and 32 outbreaks attributed to recreational water. The disease agent was identified in 41 of the 49 outbreaks. More than 4,166 became ill, and five people died. Disease organisms ranged from *E. coli* O157:H7 to the parasite *Cryptosporidium*; *Naegleria fowleri* caused four fatal cases of meningoencephalitis. Undoubtedly, many other outbreaks went unreported.

When an outbreak occurs, expertise and communication must exist to recognize it as such. An outbreak both demands immediate action to limit the outbreak, identify and treat the ill, and all possible steps to prevent a similar outbreak from occurring. It also offers public health workers and microbiologists an opportunity. During an outbreak, such as when 367 people across the United States became ill from shellfish poisoning, medical personnel often have the disease organism in hand. This is an opportunity to genetically amplify it, sequence it, study it, and compare it to other virulent pathogens. In addition, health workers should be learning all they can from disease outbreaks for data that can feed into the risk assessment framework. CDC and EPA attempt to use their surveillance programs for a number of things, including determining why the outbreaks occurred; establishing the disease agent and its epidemiology; training public health personnel to detect and investigate other outbreaks; and collaborating with local, state, federal, and international agencies on initiatives to prevent waterborne diseases. They use surveillance data to identify major deficiencies in providing safe drinking and recreational water. However, in most outbreaks, it is rare that all these areas are adequately covered.

One of the problems with the current national outbreaks reporting system is the lack of details recorded during the investigation. The location of the plant intake or well involved should be identified by Global Positioning System. The exact timing of the outbreak (starting and ending dates) should be recorded. In most outbreaks, the source of contamination and, often, the etiological agent are not identified. Both EPA and CDA should make outbreak investigations a high priority.

These limited and passive efforts do provide a national database of reported waterborne disease. But across the United States, utilities or agencies involved in outbreaks may not possess the in-house expertise or knowledge of molecular techniques that would be useful in identifying the source of the outbreak. To disseminate such knowledge, health workers and microbiologists need to develop a national web page that identifies general crisis management principles and provides contact information for medical and scientific resources. It could also provide information on data collection that will aid the broader purpose of risk assessment. Agencies and associations that might be involved with the development of such a site are CDC, EPA, American Water Works Associa-tion (AWWA), and Water Environment Federation (WEF).

Barriers to New Tools for Water Safety

The toolbox of new methods for detection of waterborne disease microbes is large and underutilized. These tools should replace cumbersome, inaccurate, and misleading fecal indicator tests. But change has been slow in coming. Barriers are both behavioral and practical.

Old tools are familiar and comfortable. Their databases are manageable and understood. And so there is complacency and a reluctance to try the new tools, which are not yet finely tuned. The best way to overcome these obstacles is to continue to develop and standardize the new methods, to use

them whenever possible, and to begin to build a record of success.

Limitations of different molecular methods must be acknowledged and overcome. For instance, methods that identify microbes based on their DNA, but do not determine whether those microbes are viable, present information that may be difficult to interpret and is, therefore, easily dismissed. Ambiguous answers can lead to confusion and doubts about the validity and usefulness of testing. However, viability assessment is not always necessary. For example, *Cryptosporidium* testing and surveys of water have provided valuable information despite some of the deficiencies of the test.

More people need to be trained in the new methods. In many cases, these new methods are much easier to perform. Lack of information or knowledge about them imparts a lack of confidence in what they can deliver. Inability to understand the implications of new data creates anxiety in managers and misuse of results by end users.

The regulatory framework must also encourage, rather than discourage, use of new technologies. Mandatory reporting of water testing results can lead to reluctance to test at all. Approaches to handling sensitive data and interpreting those data will assist water utilities.

RECOMMENDATIONS:

- Educate policy makers on new technologies. Professional associations and scientific societies should consider delivering this type of training.
- Improve communication between scientists and decision makers. Problems that require scientific investigation (including risk assessment) must be clearly articulated by managers and decision makers, including presentation of the context of the issue. Researchers should tailor their reports to their audience, present findings in a way that minimizes misinterpretation, and acknowledge uncertainty.
- Encourage international collaboration to evaluate new methods in a timely fashion, make recommendations, and report via the Internet. Efficacy of new methods should be evaluated against a "Gold Standard" to provide a measure of performance relative to the standard. This approach would allow users to select a method adequate for the purpose of the investigation and to evaluate their results.
- Develop mechanisms so that data collection and reporting can be done without fear of bad publicity or recrimination. Voluntary monitoring activities by water utilities should be encouraged and viewed as a service to public health and not as an invitation for outside scrutiny.

Education, Training, and Communication

Educational initiatives should target three groups:

- Training for policy makers on new technologies;
- Training of engineers, particularly civil engineers;
- Training of the water industry as a whole—from treatment plant operators to regulators.

Biomedical engineering is a relatively young and rapidly growing field. The new molecular tools available to microbiologists and engineers are continually evolving. New tools apply to microbe identification, understanding disease epidemiology and health effects, water treatment, disinfection, and environmental remediation. While young professionals are constantly entering the field, veterans must both teach and learn from the new entrants.

University educational programs are uneven. In some instances, engineers lack training in microbiology and biochemistry. On the other hand, traditional microbiologists often lack sufficient training in analytical microbiology. As universities struggle to define best teaching practices and curricula, continuing education may be provided via traveling workshops and distance education. Such courses are needed to present current information on standardization protocols, analytical methods, appropriate applications, correlation to conventional methods, and regulatory acceptance.

Critical Review and Analysis

An efficient method to develop, refine, and standardize new tools is needed. To accomplish this, we do not need to "reinvent the wheel." We can borrow concepts already in use in other areas. For example, the National Committee of Clinical Laboratory Standards (NCCLS), a global, interdisciplinary organization, has set laboratory standards worldwide. It uses a consensus approach to developing standards and guidance methods that allows all interested parties, including academic, government, and industry labs, to participate. A laboratory will initially develop a method, for instance for culturing a bacteria, and will publish its results. The method then goes into a "consensus framework." Other laboratories can try it, adding or subtracting to the original method. Laboratories post their results in an accessible arena such as the Internet. Gradually a method evolves that everyone agrees to and is applicable, interpretable, and eventually is standardized. This consensus process allows a new method to see immediate use, even as it is being improved. The NCCLS funds its operations through membership fees and from selling

guideline documents. The revenue pays experts for data and guideline review.

The same framework could be used to develop tools for managing water quality. We recommend that a number of organizations collaborate to start such a group. A consensus organization could also allow for new techniques to be validated through inter-laboratory collaboration and for testing that assesses the robustness of the technique in various environmental conditions.

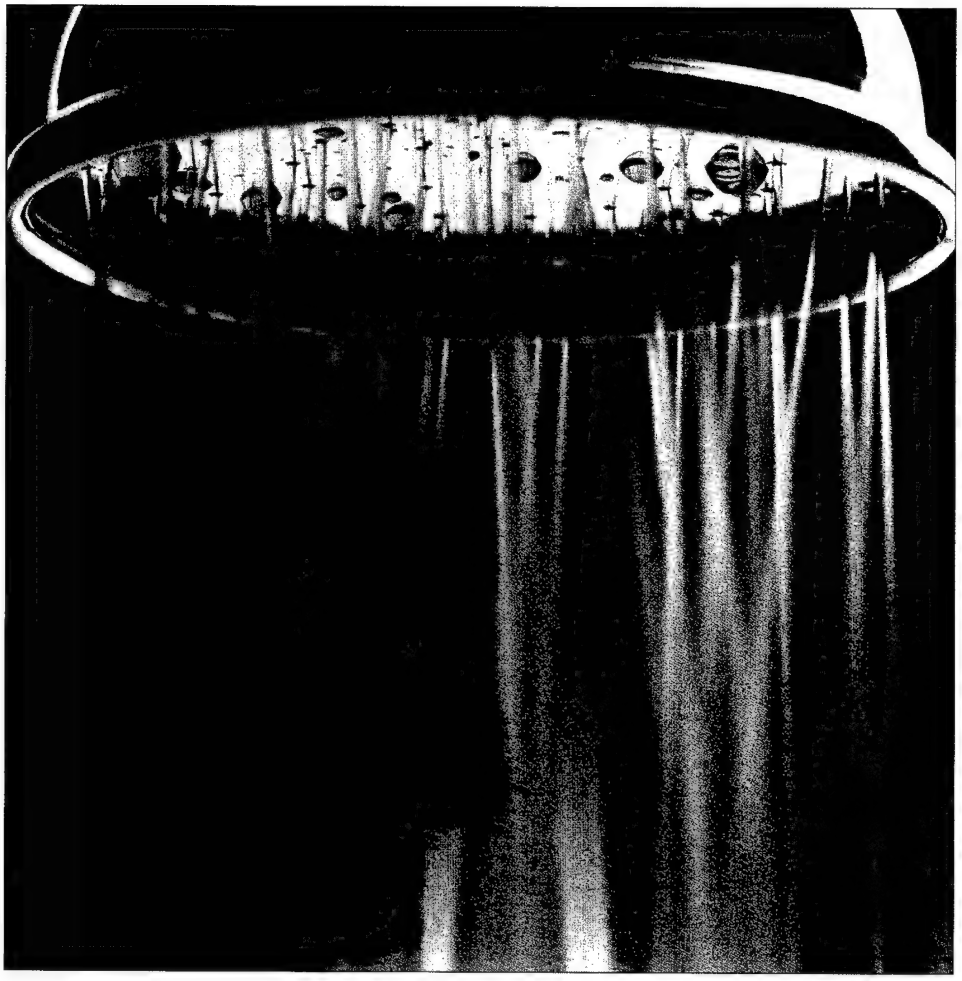
An important part of the new organization would be a National Web Site for Molecular Methods. An interactive site, it would allow individuals the opportunity to try out their methodologies using actual treatment plant data. Additionally, the site could serve as a place for authors to identify other individuals working on similar projects. The site could provide guidelines to method development, aiding in the standardization of procedures.

RECOMMENDATIONS:

- Develop a consensus organization of academic, government, and industry representatives;
- Have experts generate guidelines in a tentative and approved format to allow for sufficient review;
- Develop "test beds" to recommend and proof methods;
- "Data mine" literature to arrive at best practice recommendations.

Conclusion

Fecal coliform monitoring has led the water industry in the direction of risk reduction of waterborne diseases. These tests have been important first steps in detecting the potential for degraded waters that are unfit for human contact or consumption of shellfish and drinking water. However, repeated



experience has demonstrated the inability of the fecal coliform test to detect many harmful microbes (such as enteric viruses and parasites) found in sewage and animal wastes, which may end up in fresh surface waters, ground waters, coastal waters, and drinking waters. These tests also fail to indicate risks from naturally occurring, harmful aquatic organisms, such as toxic algae. Reliance on fecal indicators has focused risk management efforts on a system that does not properly characterize or fully understand the nature the hazards associated with water use and consumption. Thus, populations, both in the United States and internationally, remain at risk of waterborne disease.

We have been relying on outdated methods developed in the 1950s and 1960s for much too long. It is time to move to risk-based testing and regulation of our water and food supplies. By developing and applying new tools within the context of the scientific process, microbial problems in water may be detected and assessed more effectively than ever before. If these tools lay fallow as we enter the 21st century and attempt to address growing problems associated with safe drinking water, safe beaches, and safe shellfish growing waters, an escalation of water-associated health risks may be expected.

BIBLIOGRAPHY

Atmar, RL, FH Neill, JL Romalde, F LeGuyader, CM Woodley, TG Metcalf and MK Estes. 1995. Detection of Norwalk virus and Hepatitis A virus in shellfish tissues with PCR. Appl. Environ. Microbiol. 61:3014-18.

Atmar, RL, FH Neill, CM Woodley, R Manger, GS Fout, W Burkhard, L Leja, ER McGovern, F LeGuyader, TG Meltcalf and MK Estes. 1996. Collaborative evaluation of a method for the detection of Norwalk virus in shellfish tissues by PCR. Appl. Environ. Microbiol. 62:254-258.

Barwick, R, D Levy, G Craun, M Black and R Calderon. 2000. Surveillance for waterborne disease outbreaks—United States, 1997-1998. Morb. Mortal. Weekly Rep. 49(SS04):1-35.

Bej, AK. 1999. Detection of microbial nucleic acids by polymerase chain reaction in aquatic samples. In: Molecular microbial ecology manual. Adderman, ADL, J. Dirk Van Elsas and FJ De Bruijn (eds.). Kluwer Academic Publishers, Boston, MA.

Canada Communicable Disease Report. 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply. Walkerton, Ontario, May-June 2000. Vol. 26-20.

Colwell, RR, and DJ Grimes (eds.). 2000. Nonculturable microorganisms in the environment. ASM Press, Washington, D.C.

Ekins, R, and FW Chu. 1999. Microarrays: their origins and applications. Trends in Biotechnology 17:217-18.

Gilbert, L, and P Blake. 1998. Outbreak of Escherichia coli O157:H7 infections associated with a water park. Georgia Epidemiology Report 14:1-2.

Glatzer, MB. 1998. Shellfish-borne disease outbreaks in the U.S., 1992-1998. U.S. Food and Drug Administration, Southeast Regional Office, Atlanta, GA. Internal Technical Report.

Grimes, DJ. 1991. Ecology of estuarine bacteria capable of causing human disease: a review. Estuaries 14: 345-360.

Harwood, VJ, J Whitlock and V Withington. 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: Use in predicting the source of fecal contamination in subtropical waters. Appl. Environ. Microbiol. 66:3698-3704.

Hulten, K, H. Enroth, T Nystrom and L Engstrand. 1998. Presence of Helicobacter species DNA in Swedish water. J. Appl. Microbiol. 85(2):282-86.

LeGuyader, F, FH Neill, MK Estes, SS Monroe, T Ando and RL Atmar. 1996. Detection and analysis of a small round-structured virus strain in oysters implicated in an outbreak of acute gastroenteritis. Appl. Environ. Microbiol. 62:4268-72.

Lemus, J, and S. Weisberg (eds.). 2000. Huntington Beach closure investigation: technical review. 2000. USCSG-TR-01-2000 Sea Grant University of Southern California, Los Angeles, CA.

Lipp, EK, R Kurz, R Vincent, C Rodriguez-Palacios, SR Farrah and JB Rose. 2001. Seasonal variability and weather effects on microbial fecal pollution and enteric pathogens in a subtropical estuary. Estuaries. In press.

National Research Council. 1999. Setting priorities for drinking water contaminants. National Academy Press, Washington, DC.

National Research Defense Council. 1999. Testing the waters. Washington, DC.

Rippey, SR. 1994. Infectious diseases associated with molluscan shellfish consumption. Clin. Microbiol. Rev. 7:419-25.

Shieh, Y-SC, SS Monroe, RL Fankhauser, GW Langlois, W Burkhardt III and RS Baric. 2000. Detection of Norwalk-like virus in shellfish implicated in illness. J. Inf. Dis. 181 (Suppl. 2):S360-66.

Sinton, LW, RK Finlay and DJ Hannah. 1998. Distinguishing human from anbimal faecal contamination water: a review. New Zealand J. Marine Freshwat. Res. 32:323-48.

World Health Organization (WHO). 2000. The gateway to children's environmental health. www.who.int/peh/ceh/index.htm

APPENDIX

VIRUSES	NUCLEIC ACID	DISEASE(S)	WASTE(S)
Adenoviruses			
Human adenovirus	DNA	acute respiratory, pharyngitis, acute hemorrhagic cystitis	wastewater
Enteric adenovirus	DNA	gastroenteritis	wastewater
Caliciviruses			
Calcivirus	RNA	gastroenteritis	wastewater
Norwalk virus	RNA	gastroenteritis	wastewater
Coronaviruses			
Enteric coronavirus	RNA	intestinal disorders	
Orthomyxoviruses			
Influenza virus	RNA	influenza	human, swine, and fowl wastes
Picornaviruses			
Coxsackievirus A	RNA	meningitis, herpangia, common cold	wastewater
Coxsackievirus B	RNA	mycarditis, pleurodynia, rash, meningitis, paralysis	wastewater
ECHO virus	RNA	paralysis, diarrhea, meningitis	wastewater
Hepatitis A virus	RNA	infectious hepatitis	wastewater
Poliovirus	RNA	Poliomyelitis	wastewater
Reoviruses			
Reovirus	RNA	respiratory, gastroenteritis	wastewater
Rotavirus	RNA	infantile diarrhea	wastewater

APPENDIX 1. HUMAN VIRUSES FOUND IN WASTE MATERIALS THAT ENTER WATER.

SPECIES	DISEASE(S)	WASTE(S)
Acinetobacter calcoaceticus	nosocomial	water, human skin and mouth
Aeromonas hydrophila	septicemia, wound infections, diarrhea	water (fresh and estuarine)
Aeromonas sobria	septicemia, wound infections, diarrhea	water (fresh and estuarine)
Aeromonas caviae	septicemia, wound infections, diarrhea	water (fresh and estuarine)
Bacteroides fragilis	Intraabdominal abscesses	animal feces
Bacteroides melaninogenicus	orofacial	human mouth and feces
Brucella spp.	brucellosis	animal feces, urine, and milk
Campylobacter fetus	septicemia	animal feces
Campylobacter jejuni	enteritis	animal feces
Chromobacterium violaceum	septicemia and diarrhea	soil and water
Citrobacter spp.	nosocomial	water
Clostridium botulinum	botulism	soil, sediment, and fish
Clostridium difficile	pseudomembranous colitis	vagina and gastrointestinal tract
Clostridium perfringens	gangrene, wound abscesses, and food poisoning	animal feces
Clostridium sporogenes	gangrene	soil and animal feces
Clostridium tetani	tetanus	soil and animal feces
Coxiella burnetii	Q fever	milk and animal wastes
Enterobacter spp.	nosocomial	wastewater
Erysipelothrix rhusiopathiae	erysipeloid	animal feces and fish slime
Escherichia coli	gastroenteritis	wastewater
Flavobacterium meningosepticum	nosocomial, meningitis	freshwater
Francisella tularensis	tularemia	rodents and freshwater
Fusobacterium necrophorum	liver and soft tissue abscesses	wastewater and animal feces
Klebsiella pneumoniae	pneumonia, bacteremia, and nosocomial	water, feces, soil, and plants
Legionella pneumophila	Legionnaires' disease	freshwater, cooling tower water, and hot water tanks
Leptospira interrogans	leptospirosis	urine
Listeria monocytogenes	listeriosis	soil and feces
Morganella morganii	urinary tract and nosocomial	water, feces, and decaying animals
Mycobacterium tuberculosis	tuberculosis	wastewater
Mycobacterium marinum	swimming pool granuloma	water and fish
Plesiomonas shigelloides	gastroenteritis	water, fish, and aquatic animals
Proteus spp.	urinary tract and nosocomial	water, feces, and decaying animals
Pseudomonas aeruginosa	burn, wound, corneal, ear, urinary, lung, skin, and gastrointestinal tract	water, wastewater, plants, sediment, and fish
Pseudomonas pseudomallei	meliodosis	water and soil
Salmonella typhi	typhoid fever	wastewater
Salmonella enteritidis	gastroenteritis and septicemia	wastewater, animal wastes and feed, and compost
Serratia marcescens	nosocomial	water, plants, insects, and feces
Shigella boydii	bacillary dysentery	primate feces and wastewater
Shigella dysenteriae	bacillary dysentery	primate feces and wastewater
Shigella flexneri	bacillary dysentery	primate feces and wastewater
Shigella sonnei	bacillary dysentery	primate feces and wastewater
Staphylococcus aureus	abscesses and food poisoning	mammalian skin and ocean water
Streptococcus faecalis	endocarditis	animal feces
Vibrio Alginolyticus	wound infection	ocean water and aquatic animals
Vibrio cholerae	Asiatic cholera	wastewater, shellfish, and saltwater
Vibrio parahaemolyticus	gastroenteritis	saltwater and shellfish
Vibrio vulnificus	septicemia and wound infection	oysters and seawater
Yersina enterocolitica	gastrointestinal, acute mesenteric lymphadenitis	water, milk, mammalian alimentary canal

APPENDIX 2. PATHOGENIC BACTERIA FOUND IN WASTE MATERIALS THAT ENTER WATER.

APPENDIX

FUNGUS	DISEASE(S)	WASTE(S)
Aspergillus fumigatus	aspergillosis	decaying vegetation, especially grains
Candida albicans	candidiasis	animal feces
Cryptococcus neoformans	cryptococcosis	pigeon and bird feces, cellar dirt
Geotrichum candidum	geotrichosis	tomatoes, fruits, dairy products
Histoplasma capsulatum	histoplasmosis	chicken feces, bat guano

APPENDIX 3. PATHOGENIC FUNGI ASSOCIATED WITH WASTE MATERIALS THAT ENTER WATER.

SPECIES	DISEASE(S)	WASTE(S)
Cyanobacteria		
Anabaena Flos-aquae	neuromuscular poison	eutrophication and blooms
Aphanizomenon Flos-aquae	neuromuscular poison	eutrophication and blooms
Lyngbya majuscula	swimmers itch	seawater
Microcystis aeruginosa	hepatomegaly and liver necrosis	eutrophication and blooms
Oscillatoria nigroviridis	swimmers itch	seawater
Schizothrix calcicola	swimmers itch	seawater
Eucaryotic algae		
Gonylaulax spp.	paralytic shellfish poisoning	???
Gymnodinium breve	paralytic shellfish poisoning	???
Pyrodinium monilatum	paralytic shellfish poisoning	???
Gambierdiscus spp.	ciguatera seafood poisoning	???

APPENDIX 4. WASTE ASSOCIATED CYANOBACTERIA AND EUCARYOTIC ALGAE PATHOGENIC FOR HUMANS.

SPECIES	DISEASE(S)	WASTE(S)
Mastigophora (flagellates)		
Chilomastix mesnili	diarrhea?	primate feces
Giardia lamblia	giardiasis	human feces
Sarcodina (amebas)		
Entamoeba histolytica	amebic dysentery	human and other animal feces, wastewater
Dientamoeba fragilis	mild diarrhea	human feces
Naegleria fowleri	primary amebic meningoencephalitis	human feces, wastewater
Acanthamoeba spp.	amebic meningoencephalitis	human feces, wastewater, and heated water
Sporozoa		
Cryptosporidium spp.	cryptosporidiosis	animal feces
Sarcocystis spp.	sarcocystosis	animal feces
Toxoplasma gondii	toxoplasmosis	animal feces, especially cats
Ciliata		
Balantidium coli	balantidiasis	animal feces, especially swine

APPENDIX 5. WASTE ASSOCIATED PROTOZOA PATHOGENIC FOR HUMANS.

APPENDIX

SPECIES	DISEASE(S)	WASTE(S)
Digenetic trematodes (flukes)		
Schistosoma haematobium	schistsomiasis	human feces
Schistosoma japanicum	schistsomiasis	human feces
Schistosoma mansoni	schistsomiasis	human feces
Echinostoma spp.	diarrhea	animal feces
Faxciola hepatica	liver necrosis and cirrhosis	animal feces
Paragonimus westermani	paragonimiasis	animal feces and crustaceans
Clonorchis sinensis	bile duct erosion	human feces and raw fish
Heterophyes heterophyes	diarrhea and myocarditis	human feces and raw fish
Cestodes (tapeworms)		
Diphyllobothrium latum	diarrhea and anemia	human feces and raw fish
Taeniarhynchus saginatus	dizziness, nausea, pain, and inappetence	human feces and raw beef
Taenia solium	dizziness, nausea, pain, inappetence, cysticercosis	human feces and raw pork
Echinococcus granulosus	hydatidosis	dog and other animal feces
Hymenolepis nana	dizziness, nausea, pain, and inappetence	human feces
Nematodes (roundworms)		
Trichuris trichiura	asymptomatic to chronic hemorrhage	human feces
Trichinella spiralis	trichinosis	raw or undercooked meat
Strongyloides stercoralis	strongyloidiasis	human feces
Necator americanus	iron-deficiency anemia and protein deficiency	human feces
Ancylostoma duodenale	iron-deficiency anemia and protein deficiency	human feces
Ascaris lumbricoides	ascariasis	human, pig, and other animal feces

APPENDIX 5. WASTE ASSOCIATED PROTOZOA PATHOGENIC FOR HUMANS.